# Formulating with Single-Cell Proteins

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When it comes to finished ingredients, alternative protein will come in a variety of forms that perhaps can be categorized more simply  as macro- and micro- proteins. The former will derive from traditional and emerging food crops, whereas the latter will derive from traditional and emerging microorganisms.

## The bigger picture is alternative protein.

There has been a significant amount of attention brought to the topic of protein production via fermentation. The discussion has largely led with its advantages over traditional plant and animal agriculture, touched upon the bleak reality of high infrastructure costs, and sprinkled in hopes for market launch in the next one to three years. However, there has been little said to the first of its adopters -- the food scientists tasked with formulating with them. This article aims to create an accurate account of single-cell protein production to build an understanding that speaks beyond brand narratives and instead to their scientific application in food products. When it comes to finished ingredients, alternative protein will come in a variety of forms that perhaps can be categorized more simply  as macro- and micro- proteins. The former will derive from traditional and emerging food crops, whereas the latter will derive from traditional and emerging microorganisms. The emphasis here is that the transition from a predominantly macro to micro food ingredient landscape will borrow from technologies that have long existed while making strides in new innovations.

## The bigger picture is alternative protein (continued)

This article focuses on the alternative micro-protein referred to as single-cell proteins (SCP). In this context, SCP are edible microorganisms with application in human consumption (although, they are created for animal feed purposes as well). From these microorganisms (typically unicellular), its biomass or a recombinant protein type can be extracted and applied for food use. The type of microorganism may include one or a combination of strains from algae, yeasts, fungi, and bacteria. Finally, the output of its subsequent cells may be used as a finished ingredient in the production of a variety of food products -- but perhaps most immediately in protein-rich substitutes for legacy meat, egg, and dairy products.

Image 1. Brewery fermentation tanks. Though this is not exact to the infrastructure needs of SCP production, fermentation tanks are a component to their upstream production processes.

As you may reasonably conclude thus far, it is difficult to put this sector of food innovation in a box. In attempts to better understand the diversity of options that will emerge in the coming 1-3 years (and beyond), there are 5 key elements to navigate differentiation. They are:

1. Feedstock
2. Microorganism
3. Production infrastructure
4. Product
5. Market

To elaborate...

## 1. Feedstock

The feedstock, also referred to as inputs or fermentation media, will be determined in part by the type of microorganism, and will notably vary depending on its mode of metabolism (heterotrophic vs. autotrophic). This will dictate if the energy needs are derived from organic compounds, inorganic compounds, or the proportions of each.

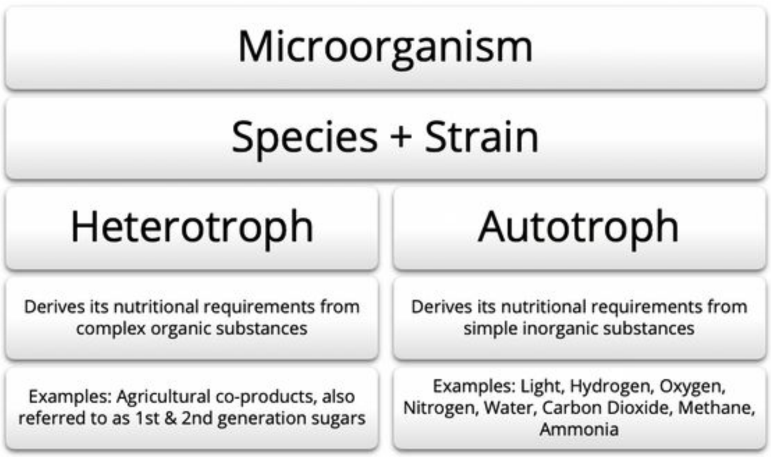


Figure 1. A microorganism is selected, more specifically, its species and strain. Based on its mode of metabolism (heterotrophic vs autotrophic), the diet of the microorganism will be either or a combination of organic and inorganic substances. Organic substances will continue a reliance on traditional plant agriculture and therefore share its inefficiencies. A long-term approach to sufficiency and reliability will look to autotrophic microorganisms.

## 2. Microorganism

Microorganisms are selected for their efficiency, which can be measured in a myriad of ways such as its biomass conversion rate, product consistency, nutritional composition, and its functional attributes. To reach the desired species and strain of that microorganism, genetic modification can be employed, although it doesn't necessarily have to. For reasons that may prioritize consumer acceptance, the desirable qualities may be reached by (1) screening for microorganisms which already have the desired trait, or (2) training the microorganisms via selective adaptation. The ultimate goal is to optimize a variety of factors that make the difference between what makes single-cell protein production theory and commercially feasible.

Within each microorganism, a gene is inserted to enable the synthesis of proteins. These proteins have amino acid sequences identical to the source of origin (which may be a plant or animal derived). To elaborate, genes are a stretch within a DNA molecule that code for something. In this context, the genes communicate how to make a protein or a group of proteins. A gene can be taken from a plant or animal cell, and inserted into the DNA of another cell. From there, the gene will be read and produce the sequenced protein. Genes can be short or long stretches of DNA, and its length and sequence will ultimately determine the function of the protein.

## 2. Microorganism (continued)

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## 3. Production Infrastructure

Production infrastructure's upstream processes and equipment may look very similar across competitors in the single-cell protein category.

Pure culture of the microorganism strain is submerged into fermentation media that proliferates and transitions from a seed fermentor (or series of seed fermentors) to a production fermentor.

The downstream process is likely to vary given its high specialization and combination of the following components:

Centrifugation
Flotation
Precipitation
Coagulation
Filtration
Dehydration

The goal of downstream processing is effective recovery of the target nutrient (which may be cell biomass or recombinant protein) and that its recovery yields as high a level of purity as possible. Throughout the course of the above processes, chemical control measures are taken, microbiological analyses conducted to ensure the absence of foreign materials, and the production strain's identity confirmed.

Here are the specifics of the three differentiators mentioned thus far, using Perfect Day as an example:

*Trichoderma reesei* strain (fungi) is selected (*Trichoderma reesei* QM6a-PD1) and proliferated in fermentation tanks containing strain-specific media (feedstock). The individual *T. reesei* cells excrete Beta-lactoglobuin (a whey protein molecule derived from a domestic cow, *Bos taurus*) into the fermentation media. Recovery of the Beta-lactoglobulin is achieved through a combination of centrifugation, pH adjustment, filtration, and spray drying.

## 4. Product

Single-cell proteins are extracted from the fermentation media and undesirable cell components. As mentioned, extraction is a multi-step process that works toward increasing levels of the targeted protein's purity. What may result is a dehydrated powder of uniform particle size which can vary in color, aroma, nutritional content, and function. It is likely, especially in the case of targeting a specific protein, that purity levels are at and above 90% on a DMB.

Ingredient declarations will prove interesting, and I can envision versatility of use with either the protein molecule (Beta-lactoglobulin) or "non-animal" and the common name of the protein (in this case, non-animal whey protein), as Perfect Day did when launching their three flavors of ice-cream in July of 2019.

## 5. Market

Market can refer to a number of things, but as it relates to the needs of food scientists, I am curious what marketing call-outs and organizational certifications lay ahead for this category of ingredients. While this area is largely in the wheelhouse of a company's marketing department, it does dictate the ingredients that can and cannot comply with a product labeled USDA Organic, Non-GMO, Kosher, Halal, or allergen free. I am optimistic that in the process of seeking compliance with any of the aforementioned, conversations will have to dig deep to understand the justifications of current criteria and support an evolution like that of Standards of Identity.

Though it may be some time before compliance is certain of major consumer-oriented call-outs, it is likely that customized versions (separate from what is regularly in-stock or produced) will be made available that meet these brand requirements. In which case, it will be interesting to know if of the advantages of single-cell protein production is lower MOQs than what is the reality for many plant-derived protein ingredients.

## Formulation

When single-cell proteins arrive in a lab near you, specification sheets and all, I imagine the first cycle of their adoption to be a delicate balance of protein content, functionality, and taste that will still rely heavily on plant proteins for the animal-free product market. I believe the high product purity will lend itself favorably to less being required to reach either protein content goals or a quality amino acid composition, and I trust the price will be high initially. With that said, the bulk of protein content may stem from plant-derived protein concentrates and isolates, and the recipe will get as close to perfect as possible before having to add "just enough" SCP to make up for the compromised function that plants alone cannot yet deliver.

## Formulation (continued)

The beauty of this is that as SCP competes with the prices of plant proteins, it will drive greater incentive to plant protein manufacturers to invest in value-added solutions for their high volumes of co-products, namely starch. In the case it doesn't, perhaps the domination of SCP (and other fermentation-derived proteins) will occur when derivative starch (think pea, fava, chickpea, and mung) is mastered for SCP feedstock. Instead of proximity to a gas feedstock source (such as an ethanol plant), it may be as advantageous to be near a plant protein manufacturer.

As you may have sensed, the conversations to be had with technical sales reps of single-cell protein ingredients is something I daydream about. I hope the information here will help shape your questions and curiosities of this space as it continues to progress.

## Sources

It was a humbling experience to bring you this article. In what composes a mere 6-minute read, between 10-12 hours were spent combing through literature and videos. I would not have been able to write this without the help of these resources: (in order of prevalence)

- Single-Cell Protein, Wikipedia
- GRAS Notice for Non-Animal Whey Protein from Fermentation by *Trichoderma reesei*, GRAS Notices
- Strategies for Success in Single-Cell Protein Production, Lux Research
- Introduction to Fermentation, NCSU
- What is a gene?, Stated Clearly
- From DNA to protein - 3D, yourgenome